ADENOSINETRIPHOSPHATASE ACTIVITY OF AMERICAN COCKROACH [1] AND WOODROACH [2] THORACIC MUSCLE [3]

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The enzymes of mammalian tissues, particularly muscle and liver, which split inorganic phosphate from adenosinetriphosphate (ATP) have received considerable study during the past several years. Du Bois and Potter (1943) developed a method for the quantitative determination of the adenosinetriphosphatase (ATPase) of tissue homogenates and showed that it was greater in cardiac and skeletal muscles than in liver, lung, kidney, submaxillary gland, spleen, pancreas, brain, and smooth muscle of the normal rat. The ATPase in the corpora lutea of rat ovaries changes little during pregnancy and lactation (Biddulph *et al.*, 1946). More recent studies have been concerned with the kinds of ATPase found in liver and the particles with which it is associated. Kielley and Kielley (1951, 1953) and Novikoff *et al.* (1952) showed that liver mitochondria dephosphorylate ATP. A "soluble" ATPase was obtained from muscle (Kielley and Meyerhof, 1948), and from the supernatant fraction of liver (Swanson, 1951). The ATPase of yeast has been purified by Meyerhof and Ohlmeyer (1952), and that of liver by Swanson (1952). Heppel and Hilmoe (1953) separated and partially purified three different ATPases from bull seminal plasma and studied their mechanism of action on ATP. The ATPase of extracts of rabbit muscle was studied by Humphrey and Humphrey (1950). The kinetics of the dephosphorylation of ATP by rabbit myosin was investigated by Ouellet, Laidler and Morales (1952), and the mechanism of hydrolysis of ATP by lobster muscle was studied by Koshland and Clarke (1953).

The presence of ATP in the tissues of *Drosophila melanogaster* was reported by Albaum and Kletzkin (1948), and in insect muscle by Calaby (1951). The major part of the work on the enzymatic dephosphorylation of ATP by insect tissues has been done with insect muscle. Gilmour (1948) found a soluble Mg-activated enzyme in myosin extracts of grasshopper muscle which split both high energy phosphate bonds of ATP. In a further study of muscle of locusts (*Locusta migratoria* and *Castrimargus musicus*) Gilmour and Calaby (1952) demonstrated that myokinase or adenylate kinase was not responsible for the removal of the second phosphate from ATP and that the apyrase could utilize adenosine di-

[1] *Periplaneta americana.*

[2] *Leucophaea maderae.* In a previous paper the common name "woodroach" was used for this species, following the usage suggested by Scharrer (1951). Taxonomists, however, generally use the common name of "Madeira roach" for this species, as pointed out by Gurney (1953), in order to avoid confusion with native species of the genus *Parablatta,* also referred to commonly as "woodroaches."

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phosphate (ADP), inosine triphosphate (ITP), and inosine diphosphate (IDP) as substrates. Sacktor (1953) found that the ATPase of mitochondria from the thoraces of the house fly, *Musca domestica,* is activated by Mg and Mn but not by Ca ions. A Ca-activated ATPase was found in the muscle fibrils, and adenylate kinase was present in the mitochondria. Sacktor *et al.* (1953) studied the dephosphorylation of ATP by several tissues of the American cockroach, *Periplaneta americana.* These tissues were rated in the following order of decreasing activity: muscle, fat body, Malphighian tubes, nerve cord, brain, hindgut, foregut, and midgut. Mg was more effective than Ca ions in activating the ATPase of these tissues. The activity of the muscle and hindgut of the female was greater than that in these tissues of the male.

Since the catalytic breakdown of ATP by ATPase presumably supplies energy for cellular functions, it was of interest to study the ATPase system in the thoracic muscle of the American cockroach, *Periplaneta americana,* and to determine the relation of the activity of this enzyme to the age and sex of the roach. Results of preliminary studies of the thoracic muscle of the woodroach, *Leucophaea maderae,* are also reported.

Experimental Materials and Methods

The chemicals used were reagent grade and the cofactors and substrates were obtained from different sources.[4] The sodium salts of these compounds were adjusted to pH 7.4 for experimental purposes.

The roaches from which tissue was obtained were isolated at one day of age so that insects of known ages would be available for experimental purposes. A few experiments were done, however, with tissue from insects of unknown ages. The roaches were kept in glass jars and were given water and food at regular intervals.

The meso- and metathoracic muscles were removed immediately after the roaches were killed. The technique of dissecting out these tissues was described in detail in a previous paper (McShan, Kramer and Schlegel, 1954). The muscle was placed in a sharp-pointed glass homogenizer in an ice bath and homogenized in sufficient water to give a one per cent homogenate (weight of tissue in grams multiplied by 99 gives the required ml. of water).

The ATPase activity of the muscle homogenates was determined by the method reported by DuBois and Potter (1943). Three amounts of tissue were used in each determination. The controls used were the complete system without tissue, and without ATP, enabling us to make correction for the inorganic phosphate present in the reaction medium, in addition to that released by the action of the ATPase.

The constituents of the system were measured into small tubes and heated to

[4] Adenosinetriphosphate (ATP), adenosinediphosphate (ADP) and adenosine-5′-phosphate (AMP) were obtained from the Pabst Laboratories, Milwaukee, Wisconsin; glucose-1-phosphate, fructose-1-phosphate and fructose-1,6-disphosphate from the Schwartz Laboratories, Inc., New York; sodium beta-glycerophosphate and sodium phosphoglycerate from National Biochemicals, Inc., Chagrin Falls, Ohio; disodium phenylphosphate and sodium phenolphthalein phosphate from Paul-Lewis Laboratories, Inc., Milwaukee, Wisconsin; paranitrophenylphosphate from the Sigma Chemical Company, St. Louis, Missouri; and a sample of sodium phenolphosphate purified in our laboratory.

38° C. in a water bath. The proper amounts of tissue were then added to the tubes and incubation was continued for 15 minutes at which time the reaction was stopped by addition of 0.2 ml. of 50 per cent cold trichloracetic acid (TCA). The tubes were centrifuged to remove the precipitate and the amount of inorganic phosphorus in 0.3 ml. of the supernatant was determined by the method of Fiske and Subbarrow (1925) as a measure of the ATPase activity of the muscle

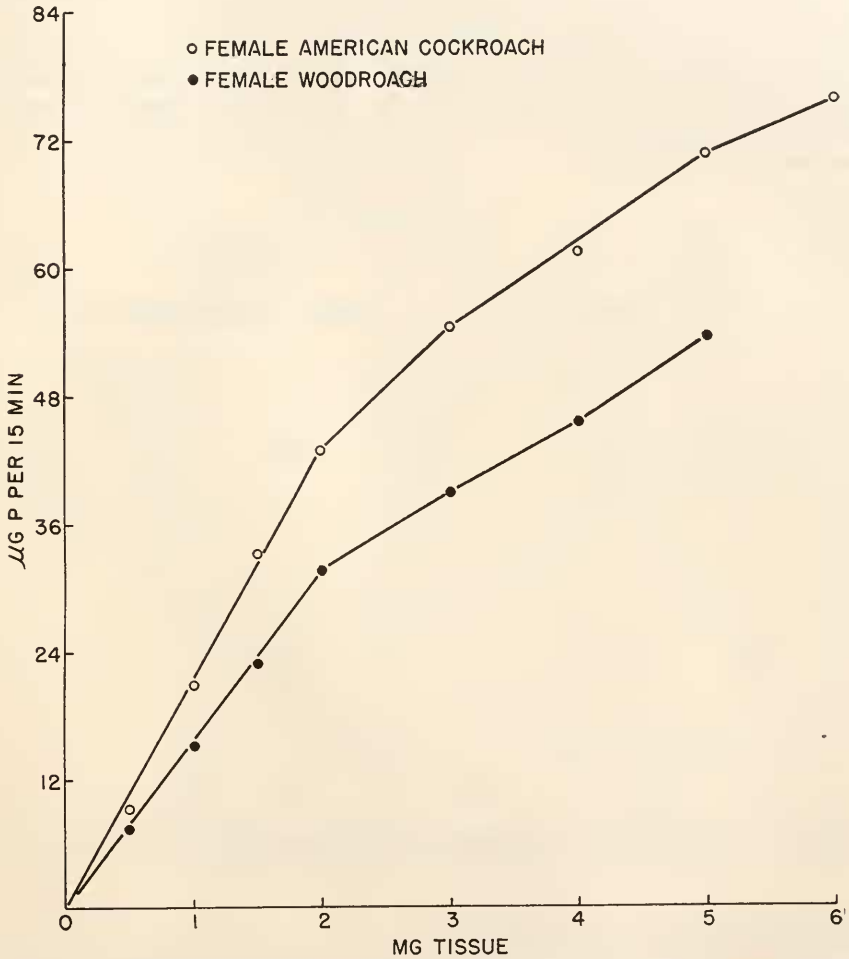

FIGURE 1. Curves showing the relations between the μg. of inorganic phosphorus released from 2.8×10^{-3} *M* ATP by different amounts of muscle tissue. The relation is linear with 0.5 mg. to 2.0 mg. of fresh tissue.

homogenate. Known amounts of phosphorus in the form of KH_2PO_4 were run with each determination as standards to serve as a basis for calculating the phosphorus content of the TCA supernatants.

One unit of ATPase activity is defined as the amount of enzyme required to release 1 μg. of inorganic phosphorus from ATP in 15 minutes at 37° C. (Du Bois

and Potter, 1943). The μg. of phosphorus released in 15 minutes by the different amounts of tissue were used for calculating the μg. of phosphorus hydrolyzed by 1 mg. of tissue. The values used for preparing the graphs and given in the tables are expressed in terms of the μg. of phosphorus released by 1 mg. of muscle in 15 minutes. These values represent the number of ATPase units per mg. of muscle.

Results

Relation of ATP concentration to enzyme activity

Maximum ATPase activity was obtained when 2 mg. of muscle tissue were incubated with as low as 1.4×10^{-3} *M* ATP. In order to insure an excess of substrate a final concentration of 2.8×10^{-3} *M* ATP was used in the system for the routine determinations.

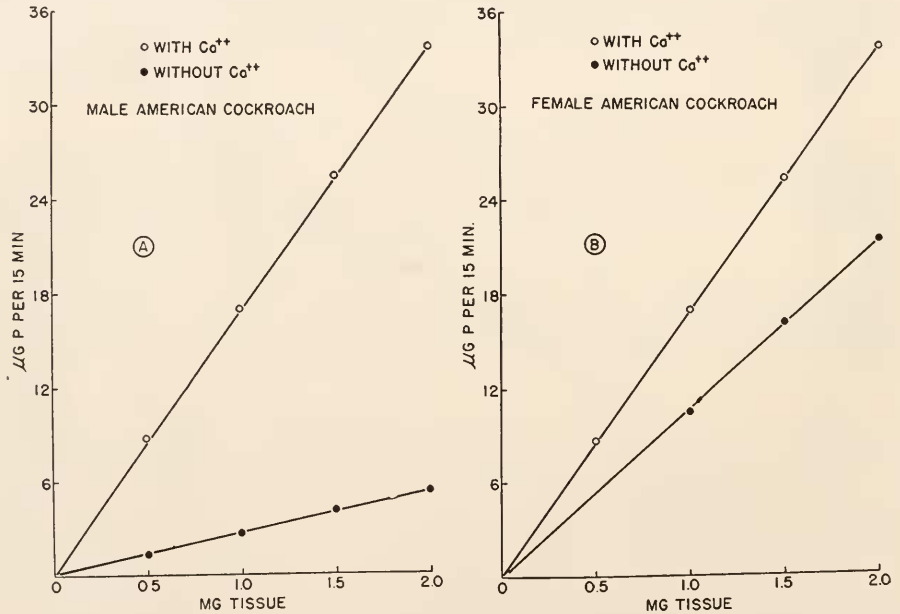

Figure 2. Effect of Ca ions on the release of inorganic phosphorus from ATP by the ATPase of muscle from the male and female cockroach.

Effect of time and enzyme concentration

The rate at which inorganic phosphorus was released from ATP by different amounts of tissue was not constant with time. This decrease in ATPase activity with time has been reported for liver homogenates (Novikoff *et al.*, 1952), for purified yeast ATPase (Meyerhof and Ohlmeyer, 1952), and for mitochondria of flies (Sacktor, 1953).

The results given graphically in Figure 1 for the muscle of the female cockroach and female woodroach show that the amounts of inorganic phosphorus released were linearly related to the enzyme concentration when 0.5, 1.0, 1.5 and 2.0 mg. of

tissue were used. This linear relation was obtained when the incubations were continued for 10, 20 and 30 minutes.

Effect of activating ions

Bivalent cations such as Ca, Mg and Mn are known to activate the ATPase of mammalian, yeast and certain insect preparations. Results represented in Figure 2A and B show that the ATPase in the thoracic muscle of the female cockroach is activated much less (58 per cent increase in activity) by Ca ions than is the enzyme of the male muscle (533 per cent increase in activity). It is also

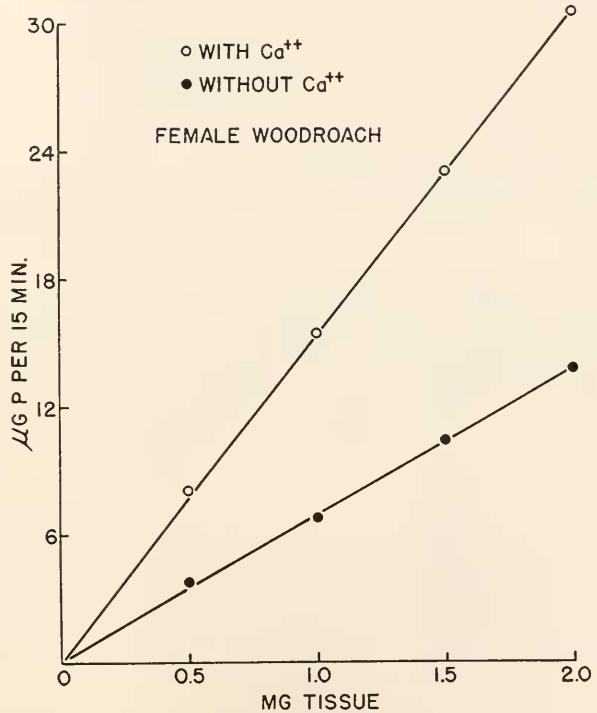

FIGURE 3. Effect of Ca ions on the release of inorganic phosphorus from ATP by the ATPase of muscle from the female woodroach.

of interest that the activity of the ATPase of muscle from the female cockroach is much greater in the absence of added Ca ions than is this enzyme in the muscle of the male. Sacktor (1953) reported results of this kind for the muscle and hindgut of male and female cockroaches. In the light of these results the question arises as to whether the greater ATPase activity of the female muscle without added Ca ions may not be due to a greater concentration of these ions in this muscle than in the muscle of the male roach.

The activation of the ATPase of muscle from the female woodroach is shown in Figure 3. The degree of Ca activation in this case is less than was found for the male but greater than that obtained for the female American cockroach.

As has been found for the ATPase of other kinds of tissue Mg and Mn ions were also found to activate the ATPase of muscle from the male and female cockroach. The degree of activation with different concentrations of Ca, Mg and Mn ions is shown in Figure 4. The ATPase activity was more nearly the same when the concentration of each of the ions was 2.8×10^{-3} *M*. Mg ions were more effective in activating the system than Ca and Mn ions at a concentration of 1.4×10^{-3} *M*. Similarly, Sacktor (1953) found that Mg ions were more effective than Ca ions at a concentration of 1×10^{-3} *M*. Ca ions were more effective in activating the ATPase of cockroach muscle at concentrations greater than 2.8×10^{-3} *M*.

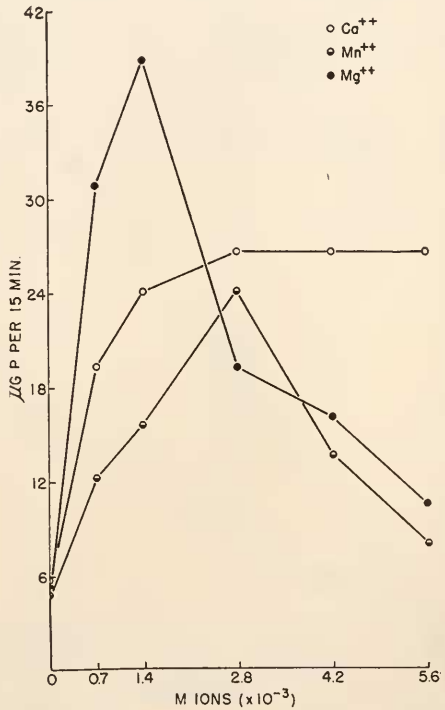

FIGURE 4. Activation of the ATPase of cockroach muscle by increasing concentrations of Ca, Mn and Mg ions.

On the basis of the above results 0.05 ml. of 0.04 *M* $CaCl_2 \cdot 2H_2O$ and 0.2 ml. of 0.01 *M* ATP (2.8×10^{-3} *M* final concentration of each) were used in the system for the determination of the ATPase of cockroach and woodroach muscle. The amounts of the other reagents used in the system were: 0.15 ml. of 0.5 *M* sodium diethylbarbiturate buffer of pH 7.4 and, as indicated above, 0.05, 0.10 and 0.15 ml. of one per cent homogenate plus sufficient water to give a total volume of 0.7 ml. for incubation. Three amounts of tissue were used for each determination to demonstrate that the phosphate released from the ATP was linearly related to the ATPase of these amounts of tissue.

TABLE I

Comparison of ATP and other phosphate esters as substrates for ATPase of male American cockroach muscle

Substrate*	ATPase Units**
Adenosinetriphosphate	15.1
Adenosinediphosphate	0.6
Fructose-6-phosphate	1.2
Glucose-1-phosphate	0.6
beta-Glycerophosphate	0.0
Phenylphosphate	0.9
Fructose-1,6-diphosphate	0.0
Adenosine-5′-phosphate	0.0
para-Nitrophenylphosphate	0.0
Phosphoglycerate	0.0
Phenolphthaleinphosphate	0.9

* The substrates were used in the form of the sodium salts and in a final concentration 0.003 *M*.
** Units per milligram of fresh muscle. Average value of three experiments using three different amounts of tissue for each experiment.

Effect of muscle homogenate on different substrates

The results given in Table I indicate that under the conditions used in these experiments the ATPase of male cockroach muscle is specific for ATP. The small amount of inorganic phosphorus released from certain of the other compounds used as substrates might be due to low activity of ATPase for these compounds, or to the action of another phosphatase.

Relation of age and sex to ATPase activity of cockroach muscle

The ATPase activity of the muscle of the American female cockroach appears to be greater than that of the male at 10, 20 and 30 days of age (Table II). This is in agreement with the results of Sacktor *et al.* (1953) who reported that the ATPase activity of female roach muscle is significantly greater than that of the male. The results of Table II also suggest that for both sexes there is a trend

TABLE II

Adenosinetriphosphatase of thoracic muscle from the American cockroach

Sex	Age, days	ATPase units*
Male	10	19.2
	20	16.9
	30	15.7
Female	10	21.6
	20	19.5
	30	18.1

* Units per milligram of fresh tissue. The values are averages of two to five determinations in which three different amounts of tissue were used. The time of incubation was 15 minutes. Ca ions were present in a concentration of 2.8×10^{-3} *M*.

toward a decrease in ATPase activity with an increase in age from 10 to 30 days. A definite conclusion on this age-ATPase activity relation is not justified, however, until determinations are made using a larger number of roaches over a wider age range.

Results of preliminary experiments on the thoracic muscle of the woodroach of various ages suggest that the sex difference in ATPase activity of the American cockroach does not exist in the woodroach, *Leucophaea maderae*. In this connection it should be mentioned that the succinoxidase activity of muscle from male woodroaches is essentially the same as the activity of muscle from the female, whereas the activity of this system in male American cockroach muscle is three to four times that of the female.

Lawrie (1952), in a study of the biochemical differences of red (high in myoglobin content) and white skeletal muscle (low in myoglobin content) of various vertebrates, indicated that in general high myoglobin content in muscle is associated with high enzymatic activity for succinic dehydrogenase, succinoxidase and cytochrome oxidase. ATPase activity, on the other hand, decreases with an increase in myoglobin content. It is interesting to note that the male American cockroach has red thoracic muscles and shows higher succinoxidase activity and lower ATPase activity than these muscles in the female which are white. There is no evidence for the presence of myoglobin in insect muscle. Sacktor *et al.* (1953), however, have suggested that in insects the difference in color may be due to the cytochrome content. Whatever the color difference may be due to, it should be noted that in the woodroach, *Leucophaea maderae*, in which both sexes have red muscles, succinoxidase and ATPase activity are apparently equivalent in both sexes.

Summary

1. The adenosinetriphosphatase (ATPase) activity of the thoracic muscle of the American cockroach, *Periplaneta americana*, and the woodroach, *Leucophaea maderae*, was studied. The optimum conditions for eliciting maximum ATPase activity of the muscle of these two species are: 0.2 ml. of 0.01 *M* ATP, 0.05 ml. of 0.04 *M* $CaCl_2 \cdot 2H_2O$, 0.15 ml. of 0.5 *M* diethylbarbiturate buffer of pH 7.4, and 0.05, 0.10 and 0.15 ml. of one per cent homogenate.

2. The ATPase activity of the muscle from females was activated less by calcium than was that of the male American cockroach. The degree of calcium activation of the ATPase of the female woodroach muscle was intermediate between that of the male and female cockroach muscle.

3. The ATPase activity of muscle from female American cockroaches 10 to 30 days of age was greater than that of male roaches of the same ages. Present results suggest that there is not a difference in the ATPase activity of muscle from male and female woodroaches.

LITERATURE CITED

Albaum, H. G., and M. Kletzkin, 1948. Adenosinetriphosphate from *Drosophila melanogaster*. *Arch. Biochem.*, **16**: 333–337.

Biddulph, C., R. K. Meyer and W. H. McShan, 1946. Adenosinetriphosphatase activity of lutein and ovarian tissues and weight of corpora lutea during the reproductive cycle of the rat. *Endocrinology*, **38**: 358–367.

CALABY, J. H., 1951. Adenosinetriphosphate from insect muscle. *Arch. Biochem. and Biophysics,* **31**: 294–299.

DUBOIS, K. P., AND V. R. POTTER, 1943. The assay of animal tissues for respiratory enzymes. III. Adenosinetriphosphatase. *J. Biol. Chem.,* **150**: 185–195.

FISKE, C. H., AND Y. SUBBAROW, 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.,* **66**: 375–400.

GILMOUR, D., 1948. Myosin and adenylpyrophosphatase in insect muscle. *J. Biol. Chem.,* **175**: 477–478.

GILMOUR, D., AND J. H. CALABY, 1952. The magnesium-activated apyrase of insect muscle. *Arch. Biochem. and Biophysics,* **41**: 83–103.

GURNEY, A. B., 1953. Distribution, general bionomies, and recognition characters of two cockroaches recently established in the United States. *Proc. U. S. Nat. Mus.,* **103**: 39–56.

HEPPEL, L. A., AND R. J. HILMOE, 1953. Mechanism of hydrolysis of adenosinetriphosphate. *J. Biol. Chem.,* **202**: 217–226.

HUMPHREY, B. A., AND G. F. HUMPHREY, 1950. The breakdown of adenosinetriphosphate in extracts of rabbit muscle. *Biochem. J.,* **47**: 238–244.

KIELLEY, W. W., AND O. MEYERHOF, 1948. Studies on adenosinetriphosphatase of muscle. II. A new magnesium-activated adenosinetriphosphatase. *J. Biol. Chem.,* **176**: 591–601.

KIELLEY, W. W., AND R. K. KIELLEY, 1951. Myokinase and adenosinetriphosphatase in oxidative phosphorylation. *J. Biol. Chem.,* **191**: 485–500.

KIELLEY, W. W., AND R. K. KIELLEY, 1953. A specific adenosinetriphosphatase of liver mitochondria. *J. Biol. Chem.,* **200**: 213–221.

KOSHLAND, D. E., AND E. CLARKE, 1953. Mechanism of hydrolysis of adenosinetriphosphate catalyzed by lobster muscle. *J. Biol. Chem.,* **205**: 917–924.

LAWRIE, R. A., 1952. Biochemical differences between red and white muscle. *Nature,* **170**: 122–123.

MCSHAN, W. H., S. KRAMER AND V. SCHLEGEL, 1954. Oxidative enzymes in the thoracic muscles of the woodroach, *Leucophaea maderae. Biol. Bull.,* **106**: 341–352.

MEYERHOF, O., AND P. OHLMEYER, 1952. Purification of adenosinetriphosphatase of yeast. *J. Biol. Chem.,* **195**: 11–17.

NOVIKOFF, A. B., L. HECHT, E. PODBER AND J. RYAN, 1952. Phosphatases of liver. I. The dephosphorylation of adenosinetriphosphate. *J. Biol. Chem.,* **194**: 153–170.

OUELLET, L., K. J. LAIDLER AND M. F. MORALES, 1952. Molecular kinetics of muscle adenosinetriphosphatase. *Arch. Biochem. and Biophysics,* **39**: 37–50.

SACKTOR, B., 1953. Investigations on the mitochondria of the house fly, *Musca domestica.* I. Adenosinetriphosphatases. *J. Gen. Physiol.,* **36**: 371–387.

SACKTOR, B., G. M. THOMAS, J. C. MOSER AND D. I. BLOCH, 1953. Dephosphorylation of adenosinetriphosphate by tissues of the American cockroach, *Periplaneta americana. Biol. Bull.,* **105**: 166–173.

SCHARRER, B., 1951. The woodroach. *Sci. Amer.,* **185**: 59–62.

SWANSON, M. A., 1951. Phosphatases of the liver. II. The so-called adenosinetriphosphatases. *J. Biol. Chem.,* **191**: 577–590.

SWANSON, M. A., 1952. Phosphatases of the liver. III. "Neutral" pyrophosphatase. *J. Biol. Chem.,* **194**: 685–693.